IN-SITU FEASIBILITY STUDY OF FRESHWATER MUSSEL REINTRODUCTION: SURVIVAL AND GROWTH OF THE SLIPPERSHELL (*ALASMIDONTA VIRIDIS*) IN THE UPPER OCONALUFTEE RIVER, NC (SWAIN CO.)

A thesis presented to the faculty of the Graduate School of Western Carolina University in partial fulfillment of the requirements for the degree of Master of Biology

By

Michelle Stacey Ruigrok

Director: Dr. Thomas Martin
Associate Professor of Biology
Biology Department

Committee Members: Dr. Karen Kandl, Biology
Mike LaVoie, Eastern Band of the Cherokee Indians

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Lastly, I dedicate this thesis to Ezra Gardiner and mussel #488.

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ABSTRACT

IN-SITU FEASIBILITY STUDY OF FRESHWATER MUSSEL REINTRODUCTION: SURVIVAL AND GROWTH OF THE SLIPPERSHELL (*ALASMIDONTA VIRIDIS*) IN THE UPPER OCONALUFTEE RIVER, NC (SWAIN CO.)

Michelle Stacey Ruigrok, M.S.

Western Carolina University (April 2019)

Director: Dr. Thomas Martin

North American freshwater mussels are an imperiled group of organisms, with 29 of the 102 species in the Tennessee River basin listed as federally endangered or threatened, and another 11 believed to be extinct (Fraley 2002). The Slippershell mussel (*Alasmidonta viridis*) has a widespread distribution but is protected as an endangered species in North Carolina. I monitored survival and growth of juvenile *A. viridis* in enclosures placed in the upper Oconaluftee River near Cherokee, NC to determine if the species may be successfully reintroduced on Eastern Band of Cherokee Indians tribal land. I also compared two enclosure designs: concrete enclosures modified from a design originally described by Chris Barnhart, and mesh enclosures that allow access to the substrate, modified from a design used by Rachael Hoch. Timed snorkel surveys were also conducted to confirm the presence of the appropriate fish host, Mottled Sculpin (*Cottus bairdii*). Between March and September of 2018, *Alasmidonta viridis* experienced significant mortality at all sites and in both enclosure designs. There was also no evidence of growth among survivors. *Alasmidonta viridis* may be sensitive to handling, as the cohort of mussels used in this study experienced increased mortality after tagging in captivity and prior to the experiment.

INTRODUCTION

The North American continent has the richest species diversity of freshwater mussels, with about 300 taxa (Haag and Warren 1998; Haag and Williams 2014). Freshwater mussels in general are declining, having extinction and imperilment rates that are among the highest of any taxa in the world. Over the past 100 years, 30 taxa in North America have become extinct, and 65% of the remaining species are classified as endangered, threatened, or vulnerable (Haag and Williams 2014).

There are 102 species of freshwater mussel in the Tennessee River basin. Twenty-nine of these species were listed as federally endangered or threatened in 2002, and 11 species may be extinct. Other species are of special concern, and only 36 of 102 species from this drainage were considered stable in 2002 (Fraley 2002). For comparison, according to the U.S. Fish & Wildlife Service's Imperiled Aquatic Strategy (2014), 24 species of freshwater mussel in the Upper Tennessee River Basin (upstream of the confluence of the Tennessee River with the Sequatchie River) – or 29% of the historic fauna – were listed as federally endangered (US Fish & Wildlife Service 2014).

Habitat destruction is the greatest threat to freshwater mussels, primarily resulting from dams and impoundments which modify habitat upstream and downstream, dredging, channel modification, siltation and contaminants, and invasive mollusks such as the zebra mussel (Williams et al. 1993; Vaughn 2010). Freshwater mussels tend to be vulnerable to disturbance and slow to recover due to their often-long generation lengths, complex reproductive cycle, low juvenile survival and colonization rates, delayed reproductive maturity, and sedentary nature (Vaughn 2010). In addition, common species are declining, so the threats to freshwater mollusks are not limited to rare species alone (Vaughn and Hakenkamp 2001). There are so many threats

to freshwater mussels that it can be difficult to properly diagnose the true cause for a population's decline and decide on the best course of action (Strayer et al. 2004).

Freshwater mussels are an important part of freshwater systems. The shells of living and dead mussels may serve as locations for algal colonization, anchoring a food source for other freshwater organisms such as benthic macroinvertebrates (Vaughn and Hakenkamp 2001). The interstitial spaces among the shells provide habitat and refugia in the benthos (Vaughn and Hakenkamp 2001; Vaughn 2010; Freshwater Mollusk Conservation Society 2016), and the accumulation of organic matter in these spaces may facilitate an increase in the abundance of certain organisms like chironomids (non-biting midges) (Vaughn and Hakenkamp 2001). They aerate substrate through burrowing, and dense mussel beds stabilize the sediment (Haag and Williams 2014; Freshwater Mollusk Conservation Society 2016).

Benthic macroinvertebrate population abundance has been observed to be greater immediately surrounding mussels or on their shells; and the composition of these invertebrates on live mussels contains more algal grazing species than does dead shells, suggesting that the presence of mussel beds influences the distribution and composition of certain freshwater fauna (Vaughn 2010). In a study comparing macroinvertebrate density in relationship to mussels, macroinvertebrate density was significantly higher in mussel beds than in patches of stream without mussels, and macroinvertebrate density was positively correlated with mussel density (Vaughn and Spooner 2006).

The filter-feeding and burrowing behaviors of mussels are important for nutrient cycling. They filter suspended solids in the water, decreasing water treatment costs and transferring nutrients from the water to the sediment, thereby depositing organic matter and excreting nutrients, which has multiple effects on the food web (Vaughn 2010; Haag and Williams 2014;

Freshwater Mollusk Conservation Society 2016). The mussels themselves are also an important source of food for fishes and other predators (Williams et al. 1993; Haag and Williams 2014), and Native Americans ate mussels and used their shells to make tools (Williams et al. 1993). Lastly, they provide pearls and their shells were used to manufacture buttons before plastic was invented (Williams et al. 1993; Strayer et al. 2004; Haag and Williams 2014). In the 1990s, shells were estimated to have a value of $40 to $50 million to the mussel shell industry (National Native Mussel Conservation Committee 1997).

Reintroduction, Captive Propagation, Habitat Restoration

Approaches to freshwater mussel conservation include habitat restoration, captive propagation, reintroduction, and translocation. The National Native Mussel Conservation Committee drafted a National Strategy for the Conservation of Native Freshwater Mussels in 1997 (National Native Mussel Conservation Committee 1997), identifying goals and strategies for nine problems related to the conservation of freshwater mussels. Goal number 8 acknowledged that "the survival and recovery of many mussel species will require the development of artificial propagation and juvenile mussel reintroduction techniques" and Goal 9 "the survival of rare mussels will require the ability to hold them in captivity or in refugia and to translocate adult mussels to reestablish populations." At the time of publication, techniques for either goal had not been perfected (National Native Mussel Conservation Committee 1997).

The updated National Strategy for the Conservation of Native Freshwater Mollusks (Freshwater Mollusk Conservation Society 2016) includes mussel restoration with the goal of abundant, self-sustaining and diverse populations, as one of nine key elements to conserve freshwater mollusks. The tone of the revised strategy is somewhat more cautious of propagation strategies. It states that propagation and translocation should be used as a last resort, citing that

habitat restoration – and efforts to limit fragmentation - have lagged behind the improvement of propagation techniques (National Native Mussel Conservation Committee 1997; Haag and Williams 2014). Haag (2012) warned that freshwater mussel conservation will be forever dependent on propagation to support small populations unless "aggressive" efforts focused on habitat are made first priority. There are cases where mussels have been propagated before sites have been identified for reintroduction, or such sites do not exist because habitat restoration was not prioritized (Haag 2012; Haag and Williams 2014).

Additionally, the rapid growth of captive propagation of freshwater mussels has raised some concerns related to population genetics, nursery stock selection, and maintaining the integrity of natural populations. A final concern is that the success of reintroduction efforts using propagated juveniles is hard to evaluate, and such evaluations have not been prioritized in many projects (Haag 2012; Haag and Williams 2014).

Restoration efforts tend to focus on the rescue of endangered species, even though evidence is building that community restoration is more likely to conserve ecosystem function (Haag 2012), and that healthy populations of common species can facilitate stable populations of rare species, because rare and common species are usually found together (Vaughn et al. 2008; Vaughn 2010; Haag 2012). Community restoration could also help stabilize populations of common species which have been shown to also be in decline (Vaughn and Hakenkamp 2001; Haag 2012; Freshwater Mollusk Conservation Society 2016). Another consideration is that low abundance of a species at a particular location is not necessarily cause for concern. Many species of mussels were rare even before modern humans started affecting their populations (Haag 2012). High mortality (Neves and Widlak 1987) and low abundance (Haag 2012) do not necessarily imply poor conditions for sustaining mussel populations. A study of juvenile mussels

in Virginia found that a mortality of 44% combined with low abundance still allowed for a viable population of mussels (Neves and Widlak 1987). The natural abundance of a species should be taken into consideration when evaluating the need for stocking (Haag 2012; Freshwater Mollusk Conservation Society 2016). Despite concerns, propagation and reintroduction are critical techniques for saving many species, and these methods have become necessary in the Tennessee-Cumberland and Mobile basins for ecological and practical concerns (Haag 2012).

Assisted colonization is a conservation approach that has not been advocated yet for freshwater mussels (Haag 2012). It is when resource managers intentionally move a species beyond its native range, either in response to – or in advance of – climate change, the lack of habitat within the species' native range (Haag 2012; Freshwater Mollusk Conservation Society 2016), or because physical barriers prevent dispersal by natural means.

Alasmidonta viridis

Range

Alasmidonta viridis (Slippershell Mussel) is a widespread species in the family Unionidae, ranging from southern Ontario to Alabama, and from South Dakota and Kansas to New York and North Carolina. Its distribution includes the Upper Mississippi River Basin; Ohio, Cumberland and Tennessee River Sub-basins; St. Lawrence River Basin; and Lake Huron, Lake St. Clair, and Lake Erie drainages (Clarke 1981; MI DNR 2009; NC WRC 2019; WI DNR 2019). In North Carolina, it is found in the Little Tennessee and the French Broad River Basins (Bogan 2002; Fraley 2002; NatureServe 2018; NC WRC 2019). It is currently found in Macon and Swain Counties near their border with Jackson County, and Henderson County, but historically had a broader range in western North Carolina (NC WRC 2019). While the Oconaluftee River is within the Little Tennessee River Sub-basin and has suitable habitat and

riverine conditions, there are no historical records of the Slippershell Mussel occurring in the river.

Habitat and Life History

Alasmidonta viridis is most commonly found in small creeks and medium-sized rivers (Clarke 1981; Fraley 2002). It follows Ortmann's Law more than other species in the genus *Alasmidonta*, where the largest and thickest specimens are found in the biggest bodies of water (Clarke 1981). The Slippershell occupies a variety of habitats across its range, such as riffle areas with larger substrate from gravel and cobble to boulder in North Carolina. In other areas, it is found in silt, sand and cobble substrate, and usually buries itself in sand or fine gravel (Clarke 1981; Fraley 2002). It can also be found in lakes and was once found in larger rivers before impoundment (Fraley 2002).

The growth of *A. viridis* is marked by concentric wrinkles and grooves in the shell and conspicuous lines showing growth intervals (Clarke 1981). Its shell, which is slightly thicker anteriorly, can grow up to 56 mm long, 36 mm high, and 23 mm wide (Clarke 1981). Its maximum lifespan is 18 years, reaching maturity at two years of age (Haag 2012). It is a long-term brooder, with spawning observed in January and February in the Little Tennessee River, and females gravid from fall to spring (Fraley 2002).

Unionid mussels release glochidia, parasitic larvae that attach to the gills of specific fish until they transform into juvenile mussels and detach from the fish, settling into the substrate. Some mussels are generalists for host fishes, while most vary in specificity (Haag and Warren 1998; Strayer et al. 2004), so host fish play an important role in mussel distribution, and in determining habitat suitability (Haag and Warren 1998; Daniel et al. 2018). The host fishes for *A.*

viridis are *Etheostoma nigrum* Rafinesque (Johnny Darter), *Cottus bairdii* Girard (Mottled Sculpin), and *Cottus carolinae* Gill (Banded Sculpin) (Clarke 1981, Fraley 2002).

Status of the Slippershell Mussel

Despite its Global Status as stable [G4G5], *A. viridis* is listed as critically imperiled [S1] in Alabama, Arkansas, Iowa, Missouri, New York [S1S2], North Carolina and Virginia. In Illinois and Wisconsin, it is listed as imperiled [S2], and threated in Michigan [S2S3] (MI DNR 2009; WI DNR 2019; NatureServe 2019). This mussel is a Federal Species of Concern (NC Biodiversity Project 2017), and Williams et al. (1993) list its conservation status as "Special Concern."

Within the tribe Alasmidontini (genera *Alasmidonta* Say (1818), *Pegias* Simpson (1900), *Arcidens* Simpson (1900), and *Arkansia* Ortmann and Walker (1912)), nine species are classified as uncommon, rare, or very rare, with three species extinct in the 20th century (Clarke 1981). North Carolina is home to five *Alasmidonta* species, four of which are listed as state endangered, and the fifth is threatened (Table 1). *Alasmidonta ravenaliana* and *A. heterodon* are federally endangered under the Endangered Species Act.

Table 1. State Rank: S1 = Critically imperiled in the state S2 = Imperiled in the state S3 = Rare or uncommon in the state S4 = Apparently secure in the state S5 = Demonstrably secure in the state. Global Rank: Global ranks replace "in the state" with "globally" (NC Biodiversity Project 2017).

Species Name	Common Name	Rank (State; Global)	Status (State, USA)
A. heterodon	Dwarf Wedgemussel	S1; G1G2	NC and USA endangered
A. ravenaliana	Appalachian Elktoe	S1; G1	NC and USA endangered
A. undulata	Triangle Floater	S3; G4	NC threatened, USA Federal Species of Concern (FSC)
A. varicose	Brook Floater	S2; G3	NC endangered, USA FSC
A. viridis	Slippershell Mussel	S1; G4G5	NC endangered, USA FSC

The federally and NC endangered *A. ravenaliana* (Appalachian Elktoe) is endemic to the upper Tennessee River System and occurs in the Little Tennessee River and French Broad River Basin in western North Carolina. Its distribution has been greatly reduced from its historic range, and now this species only occurs in stretches of the Little Tennessee, Tuckasegee, Pigeon, Nolichucky, Little, Cheoah, North Toe, South Toe, Toe and Cane Rivers (US FWS 2014; NC WRC 2019). There is a population of Elktoe in the lower Tuckasegee River below its confluence with the Oconaluftee, but there are no Elktoe in the lower Oconaluftee – below the reservoir – even though substrate and habitat appeared suitable (Fraley 2002). Through their Natural Resources program, the Eastern Band of the Cherokee Indians has invested in restoring freshwater species assemblages to their streams and rivers, including Sicklefin Redhorse (*Moxostoma sp.*). The tribe is interested in the feasibility of assisted colonization of freshwater mussels, including the Slippershell Mussel and Appalachian Elktoe, into the Upper Oconaluftee River.

Objectives

In this study, I monitored the survival and growth of juvenile *A. viridis* in protected enclosures at three locations in the Upper Oconaluftee River within the Qualla Boundary of the Eastern Band of Cherokee Indians to determine if the species may be successfully introduced on tribal land. I also compared two enclosure designs, concrete enclosures modified from a design originally described by Chris Barnhart (Barnhart et al. 2007; Huffstetler and Russ 2008), and mesh enclosures that allow access to the substrate, modified from a design used by Rachael Hoch (Hoch 2012). I included the mesh enclosure treatment because in previous projects, *A. viridis* experienced elevated mortality in concrete enclosures (NCWRC personnel, personal communication). Juvenile *A. viridis* may rely on pedal feeding to a greater extent than other

mussels and the mesh enclosures provided access to the substrate. I also conducted timed snorkel surveys to confirm the presence of the appropriate fish host, *Cottus bairdii* (Mottled Sculpin), because host fish are important in predicting mussel presence and distribution (Haag and Warren 1998; Daniel et al. 2018).

If juvenile *A. viridis* can survive and grow in the Upper Oconaluftee River, then they could potentially be a good candidate for reintroduction. Finally, if the results of the study support the reintroduction of *A. viridis*, then perhaps that could be used as justification for the assisted colonization of the federally endangered *A. ravenaliana* into the Upper Oconaluftee River.

METHODS

Study Area

The headwaters of the Oconaluftee River form at 1,611 m in elevation in the Great Smoky Mountains National Park in western North Carolina, at the confluence of Kephart Prong, Kanati Fork, and Smith Branch (Davis 2015). The Oconaluftee River, approximately 30 km in length, is a tributary of the Tuckasegee River, with a drainage area of approximately 477 km^2. Land use in the Oconaluftee River sub-watershed is 78.4% forest, 10.8% residential, 1.9% commercial, 3.1% transportation/utilities, and 5.7% other, according to the Integrated Resource Management Plan of the Eastern Band of the Cherokee Indian (EBCI 2013). The Ela Reservoir, impounded by Bryson Dam, is about 0.8 km upstream of the confluence of the Oconaluftee and Tuckasegee Rivers. Bryson Dam is a hydroelectric dam currently owned and operated by Duke Power, although it was recently purchased by Northbrook Energy. The Bryson dam was constructed in the mid-1920s and has a 0.98 MW generating capacity (Hydropower Reform Coalition 2019).

sites were selected along the Upper Oconaluftee River above Ela Reservoir (Table 2, Figure 1) within the Qualla Boundary. The Qualla Boundary, territory held as a land trust for the Eastern Band of Cherokee Indians, was established after William Holland Thomas purchased around 50,000 acres of land in Jackson, Swain, Graham and Cherokee counties for the Cherokee by 1860 (EBCI 2013). Today, the Qualla Boundary is approximately 56,698-acres in size, bordering Great Smoky Mountains National Park.

Site one was established below the wastewater treatment plant at Birdtown, Site 2 was immediately upstream of the wastewater treatment plant, and Site 3 was upstream of Soco Creek to isolate the effects from upstream development along the creek. The wastewater treatment plant

at Birdtown was updated in 2015 to treat water with extended aeration activated sludge with UV disinfection (Michael Bolt, Water Quality Section Supervisor, EBCI, personal communication). A fourth site on the Tuckasegee River was selected with the intention of using it as a control site, representative of the habitat of the Little Tennessee River drainage where the parental stock of the mussels used in the experiment were collected.

Table 2. Name, GPS coordinates, and river kilometers for the 4 sites in this study.

Site Name	River	Latitude	Longitude	River Kilometers
S1	Oconaluftee	35.457411	-83.364221	3.9
S2	Oconaluftee	35.468788	-83.350204	6.1
S3	Oconaluftee	35.468508	-83.320973	9.4
T1	Tuckasegee	35.34803	-83.238091	53.4

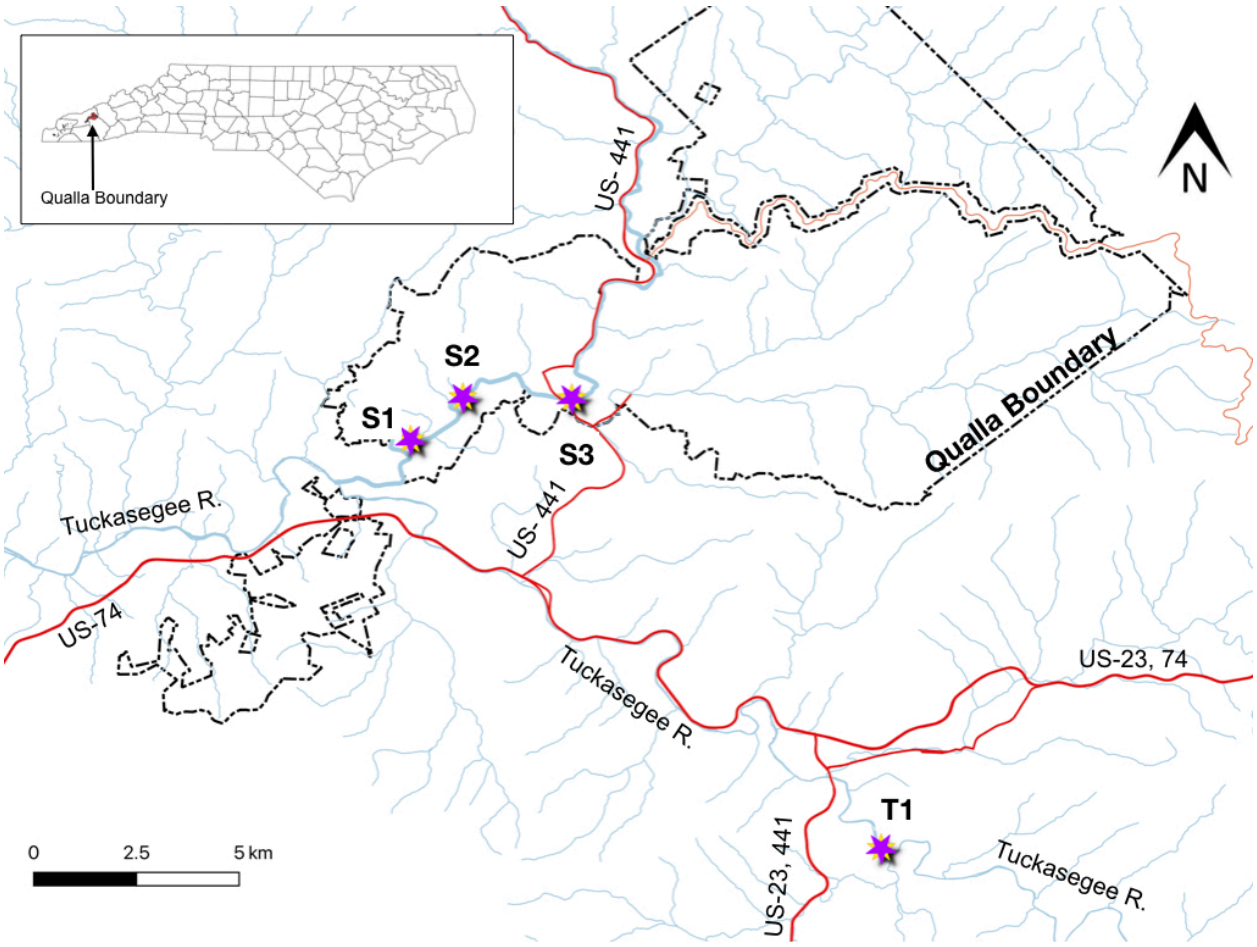

Figure 1. Map showing the locations of the 4 sites used in this study in the Oconaluftee River, Swain County, NC (S1, S2, S3) and the Tuckasegee River, Jackson County, NC (T1). All sites in the Oconaluftee River were within the Qualla Boundary of the EBCI.

Enclosures

I compared two enclosure designs, concrete enclosures modified by Virginia Department of Game and Inland Fisheries (Huffstetler and Russ 2008) from a design originally described by Chris Barnhart, Missouri State University (Barnhart et al. 2007); and mesh enclosures that allow access to the substrate, modified from a design used by Rachael Hoch for her MS thesis research (Hoch 2012).

The concrete silos (Figure 2) are designed to expose the mussels to the river environment while keeping them stable, limiting clogging by siltation, and allowing biologists to monitor their

growth rate and survivorship (Barnhart et al. 2007; Huffstetler and Russ 2008). The silo is a concrete dome with a PVC and wire mesh in the inner chamber. Water is drawn up through the inner chamber, supplying continuous fresh water and food to the mussels (Rooney 2010).

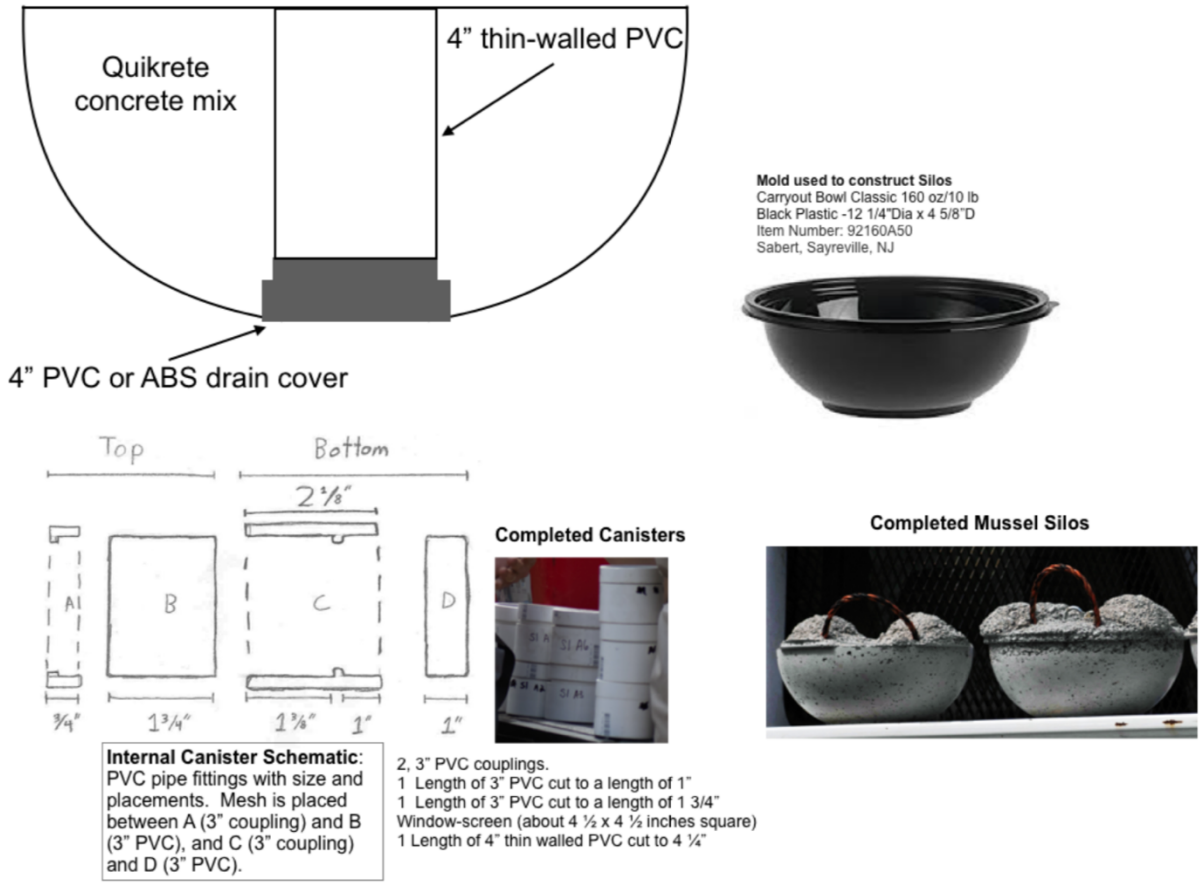

Figure 2. Concrete mussel silos were constructed from concrete and a PVC inner chamber with window screen, modified from a design by Chris Barnhart (Barnhart et al. 2007, Huffstetler and Russ 2008).

The mesh enclosures (Hoch enclosures) were 18 x 18 x 18 cm and constructed from black plastic "wildlife" fencing (3/4 x 1" mesh size) lined on the inside with window screen (Figure 3). The two layers were sewn together with plastic zip ties and the seams were sealed with 100 % silicone caulk (Hoch 2012).

Figure 3. A Hoch mesh enclosure, constructed from window screen, wildlife netting, silicone and zip ties, that has been in the river for a month.

At each site, I dug a depression into the riverbed, using the displaced substrate to half-fill the Hoch enclosures. After placing the mussels into the enclosures, I installed them in the depression and covered the three enclosures with a plastic dishwasher tray (Carlisle RF14, 45 × 50 × 10 cm, Carlisle Foodservice Products, Oklahoma City, OK) for protection from debris. The dishwasher tray at each site was anchored to the substrate with rebar and covered with a few rocks to weigh it down.

At each of the three sites on the Oconaluftee River, I installed three concrete silos and three Hoch-enclosures, with five individual mussels in each enclosure. We did not have enough *A. viridis* from the 2016 cohort to support more mussels per enclosure or to allow a fourth control site in the Tuckasegee River; however, we did place six individuals from a 2017 cohort

into one concrete silo in the Tuckasegee. I haphazardly selected individual mussels for placement into enclosures, and enclosures were installed on 3 April 2018.

Propagation

Personnel of the North Carolina Wildlife Resource Commission's (NCWRC) Conservation Aquaculture Center (CAC) in Marion, North Carolina propagated *A. viridis* from the Little Tennessee River on 20 January 2016, using *Cottus bairdii* (Mottled Sculpin) collected from Scott's Creek, Jackson County, NC as host. Brood stock had been collected from the Little Tennessee River during multiple collection efforts between 2012 and 2016. The ten females used in the propagation efforts for this study were part of a larger brood stock being held at the CAC for restoration of the Little Tennessee River drainage.

Juvenile *A. viridis* were reared in a recirculating system at the CAC with fine substrate and fed a mixture of commercially available microalgae and diatoms (Nanno 3600, *Nannochloropsis* and Shellfish Diet 1800, *Isochrysis*, *Pavlova*, *Tetraselmis*, *Thalassiosira weissflogii*, and *Thalassiosira pseudonana* from Reed Mariculture Inc., Campbell, CA). The juvenile mussels were then moved at one year of age from the recirculating system to single-pass flow through tubs supplied with coarsely filtered pond water and substrate (Rachael Hoch, CAC Coordinator, personal communication).

Tagging

Juvenile *A. viridis* in this study were used in part of another study comparing three types of tags, two glue-on style (using Loctite Super Glue Gel Control, Henkel Corp., Stamford, CT) and one laser engraving: Hallprint shellfish tags (type FPN 8x4; Hindmarsh Valley, South Australia) and The Bee Works, queen bee tags (Oro-Medonte, ON). All mussels were marked 9-10 January 2018 with the three types of tags. Those large enough to safely engrave, were given

unique identification numbers using a 30-watt, 115 v, Epilog Zing 16 laser engraver (Epilog Laser, Golden, CO) (Figure 4). Engravings and tags were coated with Omega Labs USA, Brush-On Nail Glue (San Diego, CA). Tagged mussels were measured (maximum length, width, and height) on 8 March 2018 in preparation for stocking into enclosures. Mussels from the 2017 cohort were only given queen bee tags because they were too small for Hallprint tags or laser engraving.

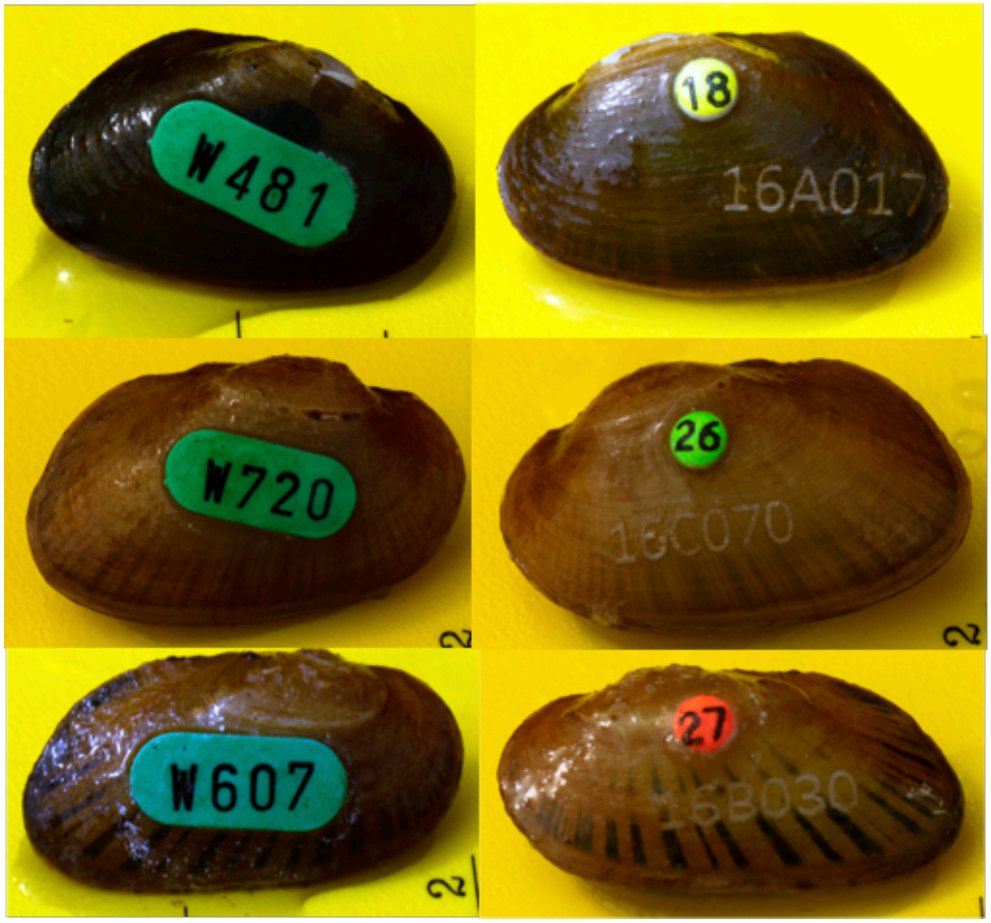

Figure 4. Tagged mussels: *Alasmidonta viridus* (top), *Lampsilis fasciola* (middle), and *Villosa iris* (bottom). Hallprint shellfish tag (left) and bee tag & laser etching (right).

Measuring Growth & Survivorship

Mussels were measured (maximum length, width and height) each month from April to November 2018 using digital calipers (with the exception of May due to equipment failure). To measure the mussels in the Hoch enclosures, the zip-ties on the lid of each enclosure were cut so that I could manually search for the mussels buried in the substrate within. Because this was a time-consuming process, mussels were immediately placed in a cooler with cold river water until all the mussels in each enclosure were found. After measuring the mussels and recording any fatalities, I removed excess debris from the enclosure, placed the mussels on top of the substrate, and sewed the enclosure shut again with zip ties. Enclosures were then returned to their original location, and any rebar that had come loose during the month was secured again using a mallet. Dead mussels were measured but not returned to the enclosure.

Water Quality

I measured nitrates, ammonia, free chlorine and phosphate each month using a HACH kit (HACH Company, P.O. Box 389, Loveland, CO 80539, USA) in case there were any changes in water quality that might affect mussel mortality or growth. These measurements were below detectable level (Nitrate 0-50mg/L, ammonia, 0-25mg/L, free chlorine 0-3.4 mg/L, and phosphate 0-50mg/L) every time so were not included in any analysis. I used a YSI meter Professional Plus Instrument (Yellow Springs Incorporated/Xylem, Inc., Yellow Springs, OH 45387, USA) to measure temperature, dissolved oxygen and conductivity. I also took velocity measurements at each site using a Swoffer 2100 meter (Swoffer Instruments, Inc., 1112 South 344th Street, Suite 302, Federal Way, Seattle, WA 98003, USA).

Snorkel Surveys for Host Fish

With the help of an assistant, I conducted timed snorkel surveys at the three sites on the Oconaluftee River on September 23, 2018 to confirm the presence of the host fish, *Cottus bairdii.* At each site, we divided the river width into three lanes. Two snorkelers conducted a single-pass transect upstream for 10 minutes, for a total of six transects per site. These data were used to augment monitoring data from the Eastern Band of Cherokee Indians which confirm the presence of *C. bairdii* in the Upper Oconaluftee River as recently as 2015. During transects, we made notes on observed instream habitat and substrate.

RESULTS

Survivorship

Alasmidonta viridis experienced high mortality after tagging in captivity at CAC and prior to this experiment. On 8 March 2018, we had 114 juvenile *A. viridis*. On 3 April 2018, when mussels were placed into enclosures in the river, only 90 individuals had survived and were available for this experiment.

There was no significant difference in survival among sites or between enclosure types (Table 3). After stocking on 3 April, 24 *A. viridis* individuals were found dead in May 2018 (Figure 5) and a total of 70 *A. viridis* were dead by our June sampling. By October, only three mussels had survived (Table 4, Figure 7).

Table 3. Summary of the test for differences in mean number of survivors per enclosure for *Alasmidonta viridis* using only the data collected in May 2018. There is no difference in survival among sites or between enclosure types.

Source	df	SS	MS	F	P
Site	21	0.0505	0.0252	0.3112	0.7383
Enclosure Type	1	0.0018	0.0018	0.0228	0.8826
Site: Enclosure Type	2	0.2741	0.1371	1.6899	0.2256
Residuals	12	0.9733	0.0811		

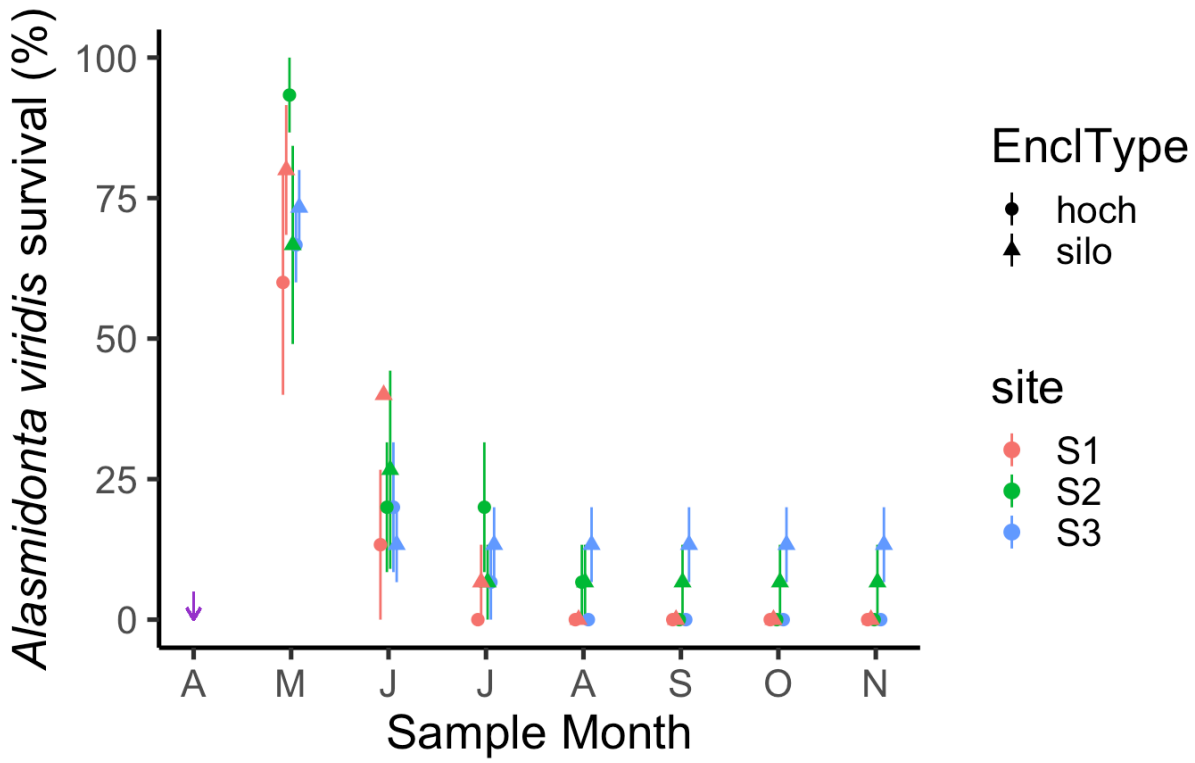

Figure 5. Mean percent survival at each sample date (there were 5 mussels per enclosure at the start of the experiment). Error bars are 1 SE. The arrow represents when the mussels were stocked into enclosures.

Growth

Mortality in the first two months of this experiment (Figure 5) was too high to be able to perform a proper analysis of growth. Only four mussels – three in concrete silos and one in a mesh enclosure - survived into August, when mussels began to show signs of growth (Figure 6). Of the surviving mussels in concrete silos, two grew between July and October, causing the mean maximum length per enclosure to produce a significant interaction: enclosure type by month (Table 5). However, I cannot conclude that enclosure type made a real difference in growth because this result is based off of so few mussels. The two mussels grew a few millimeters, and mussel #488 grew enough to exhibit an obvious growth ring between August and October (Figure 8).

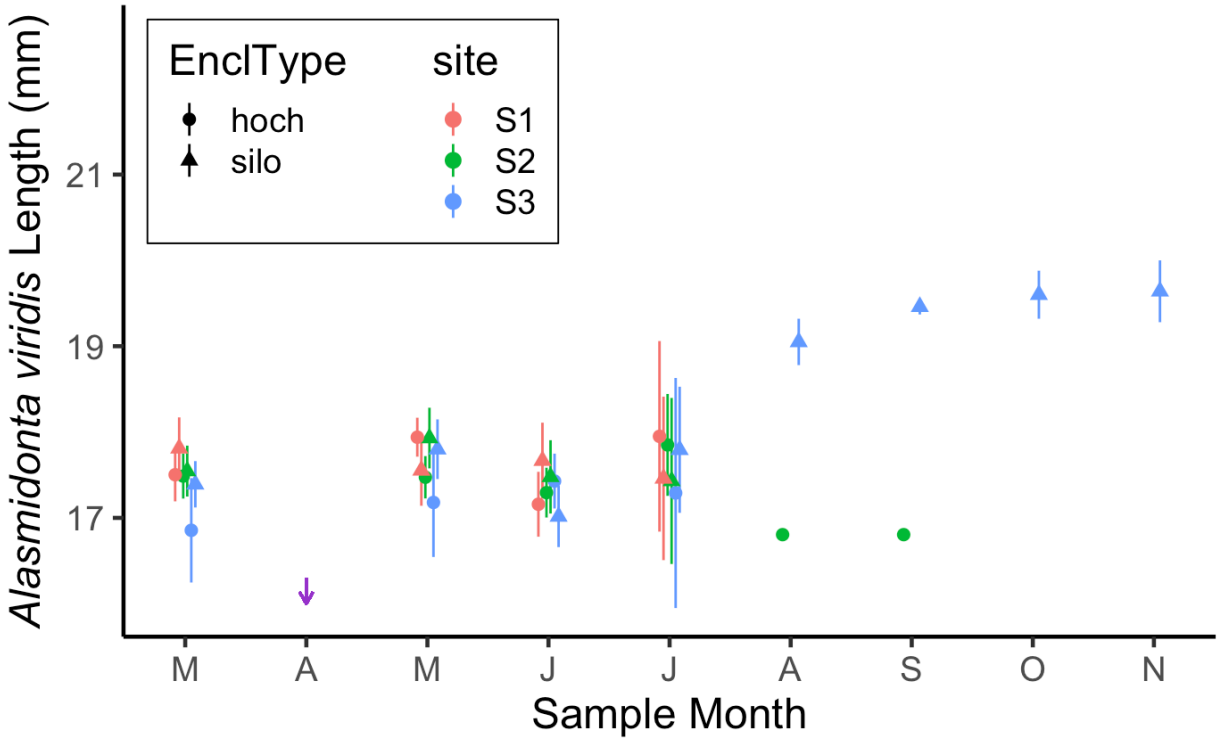

Figure 6. Mean maximum length (mm) at each sample date; error bars are 1 SE. The arrow represents when the mussels were stocked into enclosures.

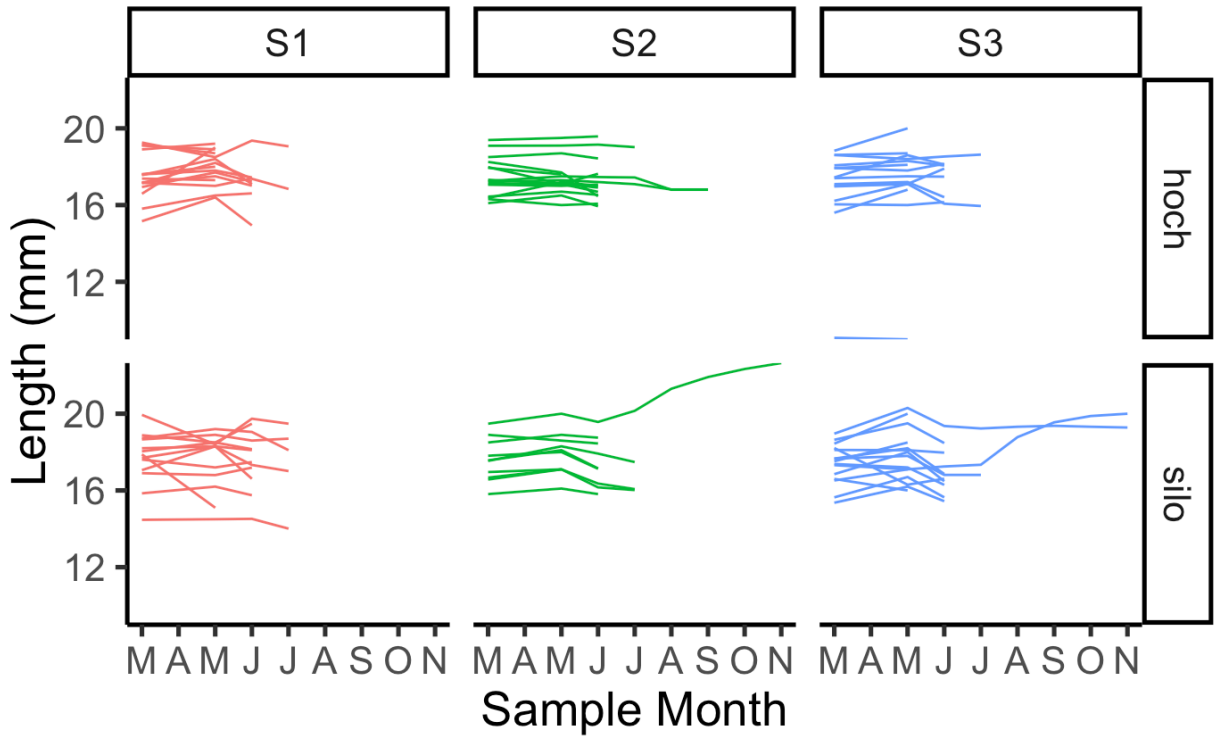

Figure 7. Lengths of individual *A. viridis* mussels in this study by site and enclosure type.

Table 4. Individual growth measurements (length (L), width (W) and height (H) in mm) for the three mussels, #482, 488 and 490 that survived into November.

Mussel Tag #	**482**			**488**			**490**		
Site		3			2			3	
Date	L	W	H	L	W	H	L	W	H
3/8/18	18.96	6.71	12.15	19.48	7.49	12.12	17.32	6.87	10.85
4/29/18	20.3	7.8	13.1	20	7.5	12.1	17.1	6.7	10.5
6/7/18	19.36	7.33	12.32	19.57	7.65	12.06	17.25	6.73	12.32
7/8/18	19.23	7.58	12.68	20.15	7.98	12.6	17.34	6.19	10.78
8/16/18	19.32	7.22	12.16	21.3	8.34	13.21	18.78	7.48	11.71
9/15/18	19.37	7.95	12.4	21.91	8.55	13.73	19.55	7.67	12.63
10/16/18	19.32	7.58	12.5	22.33	8.86	13.88	19.88	7.51	12.78
11/21/18	19.28	7.48	12.49	22.64	8.79	14.03	20	7.19	12.58
Total Growth	0.32	0.77	0.34	3.16	1.3	1.91	2.68	0.32	1.73

Table 5. Summary of repeated-measures ANOVA analyzed as a mixed-effects model for tests of differences in maximum length of *Alasmidonta viridis. A. viridis* show no evidence of growth from when they were first marked in March.

Source	SS	MS	Df_{num}	Df_{den}	F	P
Site	1.5212	0.7606	2	12.64	1.4379	0.2738
Enclosure Type	2.5437	2.5437	1	16.32	4.8091	0.0431
Month	21.1861	4.2372	5	11.02	8.0086	0.0021
Site:Enclosure Type	0.3527	0.1764	2	12.50	0.3334	0.7227
Site:Month	5.6412	0.9402	6	11.16	1.7771	0.1923
Enclosure Type:Month	9.7994	3.2665	3	11.52	6.1731	0.0095
Site:Enclosure Type:Month	2.5280	1.2640	2	12.00	2.3896	0.1338

Figure 8. Mussel #488 photographed in August 2018 (left) and October 2018 (right) showing visible growth rings.

Enclosure Comparison

I did not observe any significant difference in survivorship between the two enclosure designs (Table 3). The three individuals that survived through November 2018 were all contained in the concrete silos, suggesting that the mesh enclosures did not offer significant benefit.

Snorkel Surveys for Host Fish

Transect length varied between 28 and 56 m (Table 6). Mottled sculpin were confirmed at site 3 but not at the other sites. However, we observed more fish at each site while standing

along the banks of the river than we did during snorkeling, so *C. bairdii* may be more common at these sites than our surveys suggest. The habitat observations that we recorded are qualitative at best, so no inferences or analyses are available (Table 6).

Table 6. Snorkel transect length, location (River Right (RR), Mid-Channel (M), River Left (RL), instream habitat, and fish sightings at each site, with the target species being *Cottus bairdii* (Mottled Sculpin, MOSC).

	Site 1			**Site 2**			**Site 3**		
	T1	**T2**	**T3**	**T1**	**T2**	**T3**	**T1**	**T2**	**T3**
Length	28 m	29 m	40 m	35 m		35 m	52 m	52 m	55 m
Location	RR	M	R	RR	M	RL	RL	M	RR
MOSC								X	
Other		Darters, Dace		Northern Hogsucker, Shiners		Crayfish, Darters	White-tailed Shiner, Darter	White-tailed Shiner	White-tailed Shiner
Instream Habitat									
Pools			x						
Riffles		x		x		x			
Snags	x		x	x		x	x		x
Undercut Banks	x		x				x		x
Root Mats	x								
Backwaters									
Detritus	x		x			x	x	x	x
Aquatic Weeds		x				x			
Substrate									
Sand	x	x	x	x		x		x	x
Silt	x		x	x			x	x	x
Cobble		x	x	x		x		x	
Gravel						x			
Boulders	x	x	x	x		x			
Bedrock			x	x		x			

DISCUSSION

Survivorship

Alasmidonta viridis (Slippershell Mussels) experienced rapid mortality in this study. However, based on the observed growth of the three surviving individuals in this study, there is some evidence that *A. viridis* can grow and survive in the Oconaluftee River.

Concurrent with this study, the growth and survival *Lampsilis fasciola* Rafinesque 1820 (Wavy-rayed Lampmussel) and *Villosa iris* I. Lea 1829 (Rainbow Mussel) in concrete silos were also monitored. *L. fasciola* and *V. iris* are both imperiled in North Carolina. Growth for these two species was significant and mortality was very rare, suggesting that the conditions in the Upper Oconaluftee River are conducive for freshwater mussels (Finigan 2019). *A. viridis* was the only species in the combined study to experience significant mortality (Figure 8). Consequently, the Eastern Band of the Cherokee Indians plans to proceed with stocking and monitoring the Upper Oconaluftee River with Wavy-rayed Lampmussels and Rainbow Mussels in the next phase of this project.

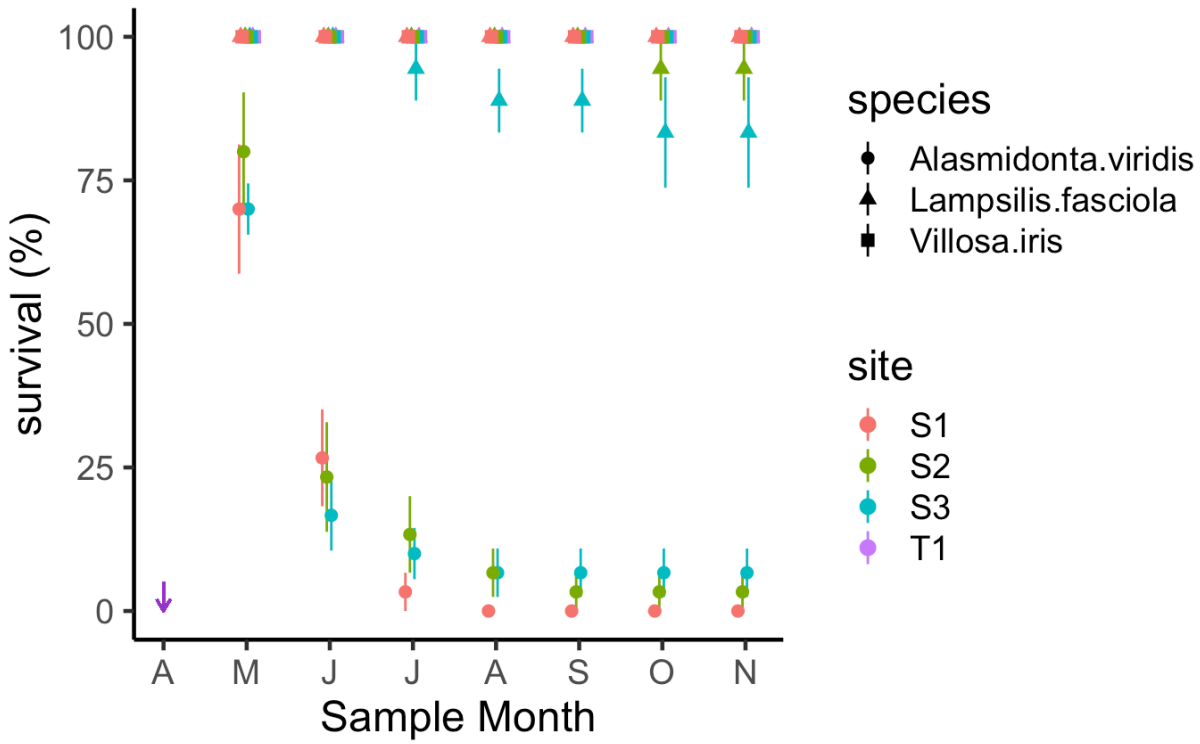

Figure 9. Percent survival of *Alasmidonta viridis, Lampsilis fasciola, and Villosa iris* each month at all sites during this study.

Handling & Hatchery Conditions. The high level of mortality of *A. viridis* and the rapid nature of the decline suggest that the mussels were experiencing stress in the hatchery and were not in ideal condition before being placed in the river. The 2016 cohort of Slippershell Mussels were experiencing declines at the NC WRC's CAC facilities prior to use in this study (Rachael Hoch, personal communication).

There are two possible explanations for these losses. The mussels likely experienced too much handling stress during the tagging, a process which involved removing the mussels from water, putting them in a machine for laser etching, coating the engraving with clear nail varnish, and allowing that to dry before returning the mussels to water. There were also two tag types that involved glue; the combined effect could be too much handling for this species of mussels. In general, staff at the hatchery try to limit disturbing and handling species in the genus

Alasmidonta, and the hall print tags were found to perform significantly better in the field than the laser tags (Finigan 2019), so I recommend only using Hallprint tags in the future.

According to CAC staff, 2018 was a bad year for mussels in the genus *Alasmidonta* at the hatchery, especially those held in the single-pass flow-through system (the "grow-out" side of the hatchery) which consists of coarsely filtered pond water and lower food availability (Rachael Hoch, personal communication). Between October 2017 and April 2018, approximately 21% (or 25 of 115 individual mussels) of the *A. viridis* at the hatchery died. Also, most of the *A. ravenaliana* (Appalachian Elktoe) that were being held in the grow-out died. At this hatchery, protocol typically calls for stocking Alasmidontines directly from the recirculating systems; however, in this case, space had to be made for the 2018 production year, so *A. viridis* and *A. ravenaliana* were transferred to the grow-out side (Rachael Hoch, personal communication).

Staff at the hatchery have also observed best growth for *Alasmidonta* species between 16˚C and 18˚C during the spring and fall months, with mortality increasing in the summer. The temperature at the hatchery climbed a few weeks before this experiment, from 15 to 19˚C, which could have stressed the mussels (Rachel Hoch, personal communication).

Cheoah River Stocking. The NC Wildlife Resources Commission has as a goal to restore priority species into the Cheoah River, a tributary to the Little Tennessee River, using captive propagation and culture (Fraley 2015). These species include *A. ravenaliana* (Appalachian Elktoe), *Lampsilis fasciola* (Wavy-rayed Lampmussel) , *Villosa iris* (Rainbow Mussel), and *Erimonax monachus* (Spotfin Chub) (Fraley 2015). The preservation of the Little Tennessee River's population of Slippershell Mussels, which is rapidly declining, is high priority for the NC WRC; as such, this species was added to the Cheoah restoration project. Slippershell Mussels from the NCWRC's CAC facility were stocked into the Cheoah River in 2013 (231

individuals), 2014 (1,296 individuals), 2015 (1,557 individuals), 2017 (1,791 individuals) and 2018 (359 individuals) (Dylan Owensby, Western Region Aquatic Wildlife Diversity Biologist, personal communication). The 359 individuals that were stocked in 2018 were from the same cohort as this study.

While it is still too early to adequately assess Slippershell Mussels from the 2018 release owing to their small size which makes them difficult to see during monitoring, Catch Per Unit of Effort (CPUE) during monitoring surveys in 2016 and 2018 were 0.03-0.6 Slippershells/person-hours and 8.0-19.3 Slippershells/person-hours, respectively (Dylan Owensby, personal communication). These data suggest that efforts to assess the feasibility of introducing Slippershell Mussels into the Upper Oconaluftee River should continue.

Growth

Mussel growth is a variable trait that depends on species, the particular population, and environmental conditions (Haag and Rypel 2011), and these environmental conditions can alter how individuals invest energy into growth (Haag 2012). Haag and Rypel (2011) stated that freshwater mussels are too often over-generalized as 'long-lived' and 'slow-growing' and found that growth and longevity among freshwater mussels can span two orders of magnitude. Conservation action must reflect the varied needs of different species (Haag and Rypel 2011); in the case of Slippershell Mussels, accounting for handling sensitivity, substrate feeding notes, and natural abundance levels. The three surviving *Alasmidonta viridis* in this experiment appear to follow the growth curve for this species (Haag and Rypel 2011). Growth for a species can even vary among populations (Haag and Rypel 2011) stressing the importance of place-based conservation and management. For the *A. viridis* in this study, we can speculate that had more

individuals survived into the cooler months, they may have showed better growth because this species appears to prefer cool temperatures (Rachael Hoch, personal communication).

Enclosure Comparison

I did not observe any significant difference in survivorship between the two enclosure designs. The three individuals that survived through November 2018 were all contained in the concrete silos, suggesting that the mesh enclosures do not offer significant benefit. These enclosures are difficult to work with in the field because the time needed to remove zip ties and then sew the enclosure shut with more zip ties after measuring the mussels. In addition, the process of searching for the mussels in the substrate is inefficient and could be another source of handling stress for the mussels. These results, however, are inconclusive due to the high level of mortality at the beginning of this study. I suggest re-designing the mesh enclosures to reduce dependence on zip ties and limit handling stress.

Snorkel Surveys for Host Fish

Of the host fishes for *Alasmidonta viridis – Etheostoma nigrum* (Johnny Darter), *Cottus bairdii* (Mottled Sculpin), and *Cottus carolinae* (Banded Sculpin), only *Cottus bairdii* occurs in the Upper Oconaluftee River, making it the focus of snorkel surveys. This species has a wide distribution in North America and is very common throughout its range, having a global status of G5 and state status in North Carolina is S5 (secure) (NatureServe b, 2019).

Snorkel surveys are commonly used to visually monitor fish populations, confirm presence/absence (as in this study), observe behavior, and more, because they are relatively inexpensive (Hankin and Reeves 1988), require minimal gear and equipment, are non-lethal and relatively non-intrusive (Weaver et al. 2014; Apperson et al. 2015). When compared with the efficiency of environmental DNA sampling, the American Fisheries Society Standard Snorkeling

Techniques were found to be more efficient (Ulibarri et al. 2017); however, snorkel surveys are less precise than other population estimation techniques, limiting their use (Mullner et al. 1998).

In a study of snorkeling efficiency by Weaver et al. (2014) conducted in the North Toe River, in Mitchell County, NC – a medium-sized river with high gradient that drains 474 square kilometers – mean snorkeling efficiency across all taxa was 14.7%, and varied from 4% to 30% depending on fish species, suggesting variation in the behavior of fish. However, the physical characteristics of a river can also dictate which sampling techniques are best – for example, seine hauls in the piedmont can have efficiencies of up to 79%, but this technique would not be appropriate in a southern Appalachian River (Weaver et al. 2014). In our study, we observed greater fish numbers from the banks of the river than we did in our snorkeling transects. Mottled sculpin was only confirmed by one individual at site 3, but our results may have been different with greater sampling effort and experience.

Conclusions

While the technology for captive propagation and release of freshwater mussels has made great advances in the past 20 years, efforts to restore or protect habitat have fallen behind (Haag 2012; Freshwater Mollusk Conservation Society 2016; Daniel et al. 2018). Conservationists also lack sufficient information regarding ideal conditions for raising *Alasmidonta viridis* and *A. ravenaliana* in captivity (Rachael Hoch, personal communication). The habitat needs of *A. viridis* should be further explored as that might help managers prioritize areas for protection (Haag 2012; Rosenberry et al. 2016; Daniel et al. 2018). Rosenberry et al. (2016) found evidence that the federally endangered *Alasmidonta heterodon* (Dwarf Wedgemussel) in Delaware are associated downstream of areas with substantial groundwater discharge, adding to the story evolving around the habitat needs and life history of mussels in the genus *Alasmidonta*.

The mortality between concrete silos (for water-column feeding) and Hoch enclosures (for substrate feeding) was not statistically different in this experiment, but the three individuals that survived into November 2018 were contained in concrete silos. Given that these mussels are commonly found buried in sand substrate (Clarke 1981; Fraley 2002), and that half of the mussels in this experiment were given access to the substrate in the Oconaluftee River yet mortality was high, further investigations are warranted into the substrate needs of *A. viridis* and the substrate conditions – such as toxicity – in the Oconaluftee River.

In habitat suitability experiments, Michaelson and Neves (1995) found that *Alasmidonta heterodon*– which shares fish hosts with *A. viridis* – in the Tar River of North Carolina preferred fine (0.063-0.850 mm) substrate, similar to *A. viridis*. A study by Archambault et al. (2014) found that temperature, water level, and thermal gradient altered burrowing behavior of mussel species. Thermal gradients in the water column are also complex and impact mussel survival (Briggs et al. 2013). Experiments are planned at the Marion Conservation Aquaculture Center to investigate the substrate needs of *A. viridis* and *A. ravenaliana* (Rachael Hoch, personal communication).

While the results of this experiment are inconclusive, several factors indicate that the Upper Oconaluftee River may be suitable for introducing *Alasmidonta viridis* into the river, including the survival and growth of three individuals, and the success of stocking in the nearby Cheoah River. When conservationists know more about the hatchery, substrate and habitat needs of *Alasmidonta viridis*, the Eastern Band of the Cherokee Indians may consider another trial study.

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